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In Silico Study of Green Tea (*Camellia sinensis*) Compound as Potential Anxiolytic Drug Material Targeting Estrogen Alpha Receptor

Harfiah Nur Aini, Susanti, Richa Mardianingrum*
Department of Pharmacy, Faculty of Health Sciences, Universitas Perjuangan Tasikmalaya, Tasikmalaya, Indonesia

*Corresponding author: richamardianingrum@unper.ac.id
Orcid ID: 0000-0002-2327-363X

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Abstract

Background: *The prevalence of anxiety disorders has significantly increased each year but has not been matched by the availability of adequate treatments. Estrogen receptor alpha (ERα) is known to induce anxiety through the activation of a complex system in the body, and drugs that inhibit ERα activity are predicted to have anxiolytic potential.* ***Objective****: This study aims to evaluate 50 compounds derived from green tea leaves to discover potential anxiolytic candidates that act by inhibiting ERα.* ***Methods:*** *The research methods used include toxicity screening, pharmacokinetic screening, drug scan, molecular docking, and molecular dynamics.* ***Results:*** *Based on the screening results, quercetin was identified as non-carcinogenic, non-hepatotoxic, easily absorbed, evenly distributed, non-interfering with CYP2D6 enzyme metabolism, and potentially effective as an oral drug. In molecular docking results, quercetin showed a ΔG value of -7.54 kcal/mol and Ki of 2.97 μM, which are better than the reference drug with a ΔG value of -7.20 kcal/mol and Ki of 5.24 μM. Quercetin also shown more stable interactions with the Erα binding site, indicated by amino acids Glu353 and Arg394 in RMSD and RMSF analysis during molecular dynamics simulation.* ***Conclusion:*** *From the study result it can be concluded that quercetin has potential as a good candidate for anxiolytic drug material by inhibiting ERα activity.*

Keywords*: anxiolytic, anxiety disorder, estrogen receptor alpha, green tea, in silico*

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INTRODUCTION

Anxiety disorders involve intense and excessive fear and worry, accompanied by physical tension and cognitive symptoms, which significantly impact daily life if untreated (WHO, 2023). As many as 301 million people worldwide were known to suffer from anxiety disorders in 2019 and that number increased significantly due to the COVID-19 pandemic, but only 27.6% of anxiety disorder sufferers received treatment due to limited availability (WHO, 2022). Meanwhile, in Indonesia, the prevalence of anxiety disorders reached 31.9% among children, adults, and the elderly with or without prior mental disorders in 2022 (Atmawati, 2022).

Estrogen alpha (ERα) is known to influence anxiety by increasing glucocorticoid secretion through the Hypothalamic-Pituitary-Adrenal (HPA) axis activity, enhancing Adrenocorticotropic Hormone (ACTH) response to stress, and regulating Corticotropin-Releasing Hormone (CRH) gene expression via histone acetylation (Borrow & Handa, 2017). Therefore, drugs that inhibit ERα receptor activity are predicted to have promising potential in treating anxiety disorders. Clonazepam, a benzodiazepine-class anxiolytic drug, has been shown through in vitro research on hERα-HeLa-99035 cells to exhibit antagonistic activity against Erα (Kenda et al., 2022).

Many studies have been conducted to discover anxiolytic drug candidates including research on green tea plants. Clinical studies indicate that consumption of decaffeinated green tea beverages positively contributes to reducing anxiety levels in adolescents who stutter (Almudhi & Gabr, 2022). *In vivo* studies also show positive effects of green tea on neurobiological behaviors, including anxiety in lead-induced mice (Al-Qahtani et al., 2022). Meanwhile, *in silico* studies were conducted to investigate the molecular interactions of green tea compounds. The ERα was chosen as the target due to its known involvement in anxiety pathophysiology through modulation of the HPA axis and stress hormone regulation.

MATERIALS AND METHODS

Materials and tools

The hardware used in this research include a Lenovo ThinkPad laptop with an Intel® Core™ i5-6200U CPU @ 2.30GHz (4 CPUs) ~2.40GHz; 8GB RAM; 64-bit system type; operating system Windows 10 Pro, and a computer with specifications featuring an Intel® Core™ i5-8400 CPU @ 2.80 GHz x 6, Nvidia Geforce GTX 970/PCIe/SSE2 GPU, 64-bit operating system type, and 245.1 GB RAM. The software utilized in this study includes MarvinSketch, Molegro Molecular Viewer, AutodockTools, Desmond, and BIOVIA Discovery Studio. Additionally, the research utilized websites such as KNApSACk, PubChem, PDBsum, RCSB PDB, and pkCSM. The materials used comprise 50 compounds found in green tea from websites http://www.knapsackfamily.com/KNApSAcK_Family/ the reference drug clonazepam, and the Erα with PDB ID 7UJM (Hosfield et al., 2022).

Method

Preparation of receptor and compound ligands

The crystallographic structure of the ligand binding domain of the Erα was downloaded from the RCSB PDB website (https://www.rcsb.org/) at a resolution of 1.80 Å (Hosfield et al., 2022). The receptor was subsequently analyzed on the PDBsum website (https://www.ebi.ac.uk/thornton-srv/databases/pdbsum/), which provides detailed receptor structure information, schematic diagrams of molecules within each structure, and their interactions with ligands (Mardianingrum et al., 2021). Further receptor preparation involved removing solvent molecules, separating the receptor from natural ligands and other residues, and adding hydrogen atoms (Mardianingrum et al., 2023).

The 50 green tea compound ligands were sourced from the KNApSAcK database (http://www.knapsackfamily.com/KNApSAcK_Family/), by entering the plant name *Camellia sinensis* (green tea) as the query. The search results were filtered to include only naturally occurring compounds reported specifically in the leaves of *Camellia sinensis*, which are commonly used in tea preparations and known to contain bioactive phytochemicals with potential pharmacological effects. Their structures are obtained from the PubChem website (https://pubchem.ncbi.nlm.nih.gov/). The compound ligands were then prepared through protonation at physiological pH conditions and conformational search using Marvin Sketch (Mardianingrum et al., 2023).

Validation of the docking method

The validation process of the docking method was conducted by redocking the ligand to the previously separated receptor. Parameters considered included the RMSD value, which is deemed acceptable if ≤ 2 Å (Mardianingrum et al., 2023). Interactions occurring between the natural ligand and amino acid residues on the receptor were then assessed based on 2D visualization. Additionally, comparisons between the

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natural ligand before and after the docking method validation process were also examined through 3D overlays.

Prediction of toxicity aspects, pharmacokinetic aspects and drug scans

The prediction of toxicity and pharmacokinetic aspects was conducted by entering the SMILES notation of the compound ligands on the pkCSM website (https://biosig.lab.uq.edu.au/pkcsm/prediction). Meanwhile, drug scanning, which refers to predicting the potential of compound ligands as orally-based drugs, was performed considering drug absorption characteristics and effectiveness through parameters such as molecular weight, log P, partition coefficient, number of hydrogen bond donors and acceptors, and molar refractivity, following Lipinski's rule of five (Mardianingrum et al., 2023).

Analysis and visualization of molecular docking results

Molecular docking was performed using AutoDockTools with the Lamarckian Genetic Algorithm (LGA) settings. Each docking was replicated three times to ensure consistency of the results. The best binding pose was selected based on the lowest binding free energy (ΔG) and inhibition constant (Ki) to proceed to molecular dynamics simulation (Mardianingrum et al., 2022). 2D visualization was conducted on natural ligands, reference drugs, and the best compound ligands from the molecular docking results to analyze amino acid residue contacts and hydrogen bond interactions (Mardianingrum et al., 2022).

Simulation and Analysis of Molecular Dynamics (MD) Results

MD were performed on the reference drug as well as on the test compound ligands using Desmond. The simulation system was set at 300^0K temperature, 1 bar pressure, 100 ns duration, with data recorded every 100 ps that resulting in a total of 1,000 frames (Lv et al., 2022). The results obtained included trajectory files analyzed based on Root Mean Square Deviation (RMSD) and Root Mean Square Fluctuation (RMSF) (Lv et al., 2022).

RESULTS AND DISCUSSION

Receptor Analysis

The 7UJM receptor is a ligand-binding domain classified as a transcription factor involved in regulating estrogen-related gene expression in Homo sapiens organisms (Hosfield et al., 2022). The receptor was analyzed using a Ramachandran plot, a statistical representation of amino acid residues in the protein structure based on φ and ψ dihedral angles (Ruswanto et al., 2018). The analysis results indicate that the 7UJM receptor meets the criteria for good structural quality, with 0.0% of residues in disallowed regions and 94.7% of residues in most favored regions, as shown in Figure 1 (a).

The binding sites of natural ligands on the receptor were analyzed using LigPlot, which automatically generates 2D schematic representations of all interactions formed between the ligand and protein molecule residues (Wadapurkar et al., 2018). Binding sites on the natural ligand are characterized by hydrogen bonds (Ruswanto et al., 2018). Therefore, based on LigPlot analysis results, it was found that the binding site of the natural ligand on receptor 7UJM involves hydrogen bonding with residues Glu353 and Arg394, as depicted in Figure 1 (b).

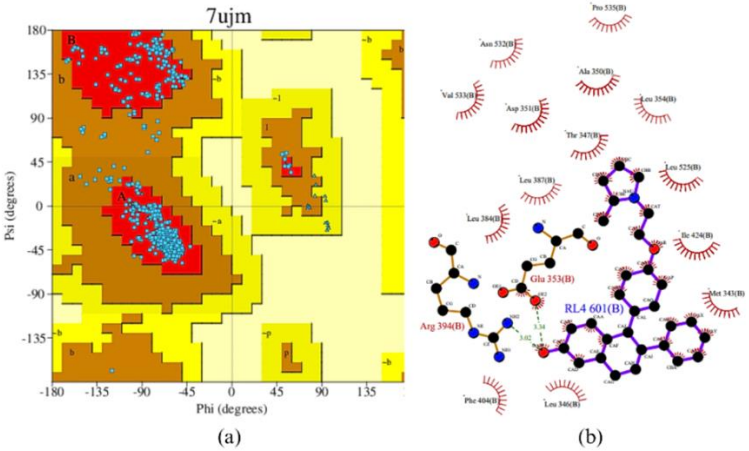

Figure 1. (a) Ramachandran statistical plot of 7UJM receptors, (b) LigPlot schematic of natural ligands on the 7UJM receptor

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Docking method validation

The docking method validation was conducted using a grid box coordinate setup that encompassed the receptor binding site area and allowed sufficient space for ligand rotation and translation (Gopinath & Kathiravan, 2021). The docking method in this study was deemed valid with an RMSD value < 2 Å because the lower RMSD value indicating the more similar docking ligand positions to the natural ligand positions from crystallography and the more effective docking method (Mardianingrum et al., 2023). Additionally, the selected receptor structure (PDB ID: 7UJM) had previously been validated through Ramachandran plot analysis, with 94.7% of residues located in the most favored regions and 0.0% in disallowed regions, confirming its structural reliability. The validation results of the docking method can be seen in Figure 2 and Table 1.

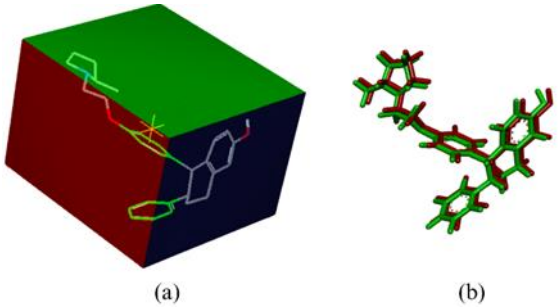

Figure 2. (a) natural ligands in a grid box, (b) 3D overlay of ligands before redocking (green) and after redocking (red)

Table 1. Validation results of the 7UJM receptor docking method along with grid box coordinate settings

Grid dimension			Grid center			Spacing	RMSD	ΔG	Ki
x	y	z	x	y	z				
30	**24**	**34**	**-0.944**	**18.643**	**18.988**	**0.375 Å**	**0.72 Å**	**-13.74 kcal/mol**	**85.02 pM**

Table 2. Prediction results of toxicity and pharmacokinetic aspects of 50 compound ligands from green tea

No	Compound name	Toxicity Aspects			Pharmacokinetic Aspects				
		Ames Toxicity	LD_{50} (mol/Kg)	Hepato-toxicity	Caco2 (Log 10^{-6})	HIA (%)	VDss (log L/Kg)	CYP2D6 inhibitor	Renal OCT2 substrate
+	Clonazepam	No	3.08	No	0.98	98.58	0.14	No	No
1.	Indole	No	2.40	No	1.54	93.30	0.26	No	No
2.	Adenine	No	2.07	No	1.37	88.74	-0.08	No	No
3.	Caffeine	No	2.80	Yes	1.11	99.27	-0.59	No	No
4.	Cytosine	No	1.94	No	0.47	83.05	-0.15	No	No
5.	Theobromine	Yes	2.38	Yes	0.51	98.37	-0.15	No	No
6.	Theophylline	No	2.30	Yes	0.62	100	0.84	No	No
7.	Xanthine	Yes	2.08	No	-0.17	63.92	-0.51	No	No
8.	Methyl anthranilate	No	1.74	No	1.18	87.46	0.01	No	No
9.	7-Methylxanthine	Yes	2.14	Yes	0.45	89.90	-0.26	No	No
10.	Paraxanthine	No	2.18	Yes	0.46	93.97	-0.24	No	No
11.	7-Methylxanthosine	No	1.93	Yes	0.63	39.33	0.04	No	No
12.	AMP	No	2.06	No	-0.66	35.80	0.57	No	No
13.	L-Theanine	No	2.06	No	-0.25	42.53	-0.56	No	No
14.	Xanthosine	No	1.85	Yes	0.19	44.83	-0.02	No	No
15.	IMP	No	2.03	No	-0.68	25.94	-0.27	No	No
16.	Xanthosine 5'-monophosphate	No	2.04	No	-0.57	22.49	-0.11	No	No
17.	7-Methyl-XMP	No	2.57	Yes	-0.97	20.89	0.37	No	No

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18.	N,N-Dibutylacetamide	No	2.03	No	1.48	93.43	0.09	No	No
19.	1-Methylxanthine	No	2.48	Yes	1.12	83.63	0.21	No	No
20.	(+)-Catechin	No	2.42	No	-0.28	68.82	1.02	No	No
21.	(+)-Epicatechin	No	2.42	No	-0.28	68.82	1.02	No	No
22.	(+)-Gallocatechin	No	2.49	No	-0.37	54.12	1.30	No	No
23.	(-)-Catechin	No	2.42	No	-0.28	68.82	1.02	No	No
24.	(-)-Epiafzelechin	No	2.36	No	1.07	91.48	0.56	No	No
25.	(-)-Epicatechin 3-O-gallate	No	2.55	No	-1.26	62.09	0.66	No	No
26.	(-)-Epigallocatechin	No	2.92	No	-0.37	54.12	1.30	No	No
27.	(-)-Epitheaflavic acid	No	2.48	2.48	-1.59	45.17	0.13	No	No
28.	(-)-Gallocatechin gallate	No	2.52	No	-1.52	47.39	0.80	No	No
29.	Catechin-3-gallate	No	2.55	No	-1.26	62.09	0.66	No	No
30.	Epicatechin 3,5-di-O-gallate	No	2.48	No	-1.25	54.04	-0.04	No	No
31.	Epicatechin 3-O-(3-O-methylgallate)	No	2.55	No	-0.27	80.84	0.71	No	No
32.	Epigallocatechin 3,3',-di-O-gallate	No	2.48	No	-1.25	39.04	0.14	No	Yes
33.	Epigallocatechin 3-O-(3-O-methylgallate)	No	2.55	No	-1.78	71.28	1.00	No	No
34.	Epigallocatechin 3-O-caffeate	No	2.55	No	-1.36	51.08	0.68	No	No
35.	Ampelopsin	No	2.43	No	0.11	58.92	1.66	No	No
36.	Astragalin	No	2.54	No	0.30	48.05	1.44	No	No
37.	Cinnamate	No	1.75	No	1.60	81.81	-1.07	No	No
38.	Cyanidin	No	2.46	No	-0.35	87.30	0.95	No	No
39.	Delphinidin chloride	No	2.54	No	-0.32	61.91	0.96	No	No
40.	Dihydroquercetin	No	2.26	No	0.92	64.70	1.63	No	No
41.	Isomyricitrin	No	2.54	No	-1.34	33.39	1.54	No	No
42.	Leucocyanidin	No	2.39	No	-0.25	56.71	1.81	No	No
43.	Naringenin	No	1.79	No	1.02	91.31	-0.01	No	No
44.	Pollenitin	No	2.36	No	-0.27	74.72	0.61	No	No
45.	Quercetin	No	2.47	No	-0.22	77.20	1.55	No	No
46.	Tricetinidin	No	2.60	No	-0.98	75.64	0.27	No	No
47.	3-O-Caffeoylquinic acid	No	1.97	No	No	-0.84	36.37	0.58	No
48.	Peonidin chloride	No	2.39	No	No	-0.04	73.69	0.62	No
49.	Malvidin	No	2.34	No	No	-0.38	88.78	0.76	No
50.	Luteoliflavan	No	2.48	No	No	0.98	90.00	0.96	No

*highlight: Not eligible

Prediction of toxicity aspects, pharmacokinetic aspects and drug scans

Prediction of toxicity and pharmacokinetic aspects was conducted using the pkCSM website based on graphical structural features (Pires et al., 2015). Parameters considered in toxicity aspects include Ames toxicity as widely used methode to assess the mutagenic potential of a compound using bacteria, rat LD_{50} as the amount of a compound that causes 50% mortality in test animal groups, and hepatotoxicity which involves chemical-induced liver damage (Pires et al., 2015). Meanwhile, parameters considered in pharmacokinetic aspects include Caco-2 permeability as an in vitro cell model that predicting oral drug absorption, intestinal absorption (human) as percentage of compound

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absorbed by the human intestine, VDss that indicating drug distribution in body tissues relative to blood plasma, CYP2D6 inhibitor as an enzyme crucial in drug metabolism, and renal OCT2 substrate that predicting compound transport potential via kidney transporters affecting drug elimination (Pires et al., 2015). Based on the results in Table 2, 35 compounds meeting the criteria for predicting both toxicity and pharmacokinetic aspects.

Drug scan was conducted on 35 compounds that had passed the prediction stage for pharmacokinetic and toxicity aspects. The drug scan stage refers to the process of scanning or analyzing test compounds using Lipinski's rule of five. Lipinski's rule of five consists of guidelines used to evaluate the pharmacokinetic properties of a compound, particularly its absorption and permeability characteristics (Chen et al., 2020). First, molecular weight < 500 Da because smaller molecules tend to be absorbed better. Second, Log P value < 5 indicating a balance between lipophilicity and hydrophilicity crucial for optimal absorption. Third, hydrogen bond donors < 5 (NH or OH groups) to minimize membrane permeability. Fourth, hydrogen bond acceptors < 10 to avoid excessive membrane permeability. Fifth, molar refractivity reflecting molecule size and polarity ideally between 40-130 (Chen et al., 2020). Based on the results of the drug scan in Table 3, 17 test compounds were found to meet all drug scan parameters. This indicates that these compounds have the potential for effective use as oral drugs, capable of being well-absorbed through the digestive tract, efficiently distributed throughout the body, and able to reach drug targets with adequate concentrations. The drug scan results can be seen in Table 3.

Analysis and visualization of molecular docking results

Molecular docking process was conducted on 17 test compounds that had passed prediction for toxicity aspects, pharmacokinetic aspects, and drug scan. The results of molecular docking were analyzed based on ΔG and Ki values. ΔG measures the ligand's ability to bind to the receptor, where more negative values indicate stronger binding affinity (Mardianingrum et al., 2022). Ki represents the affinity of the compound and its decomposition, directly proportional to ΔG, which mean the more negative the ΔG value and the Ki value, indicating more effective inhibition of ligand activity on the protein (Mardianingrum et al., 2022). The results of molecular docking can be seen in Table 4.

Based on the results of molecular docking in Table 4, two compounds were selected that showed more negative ΔG and Ki values compared to clonazepam. These compounds are (+)-epicatechin with ΔG -7.55 kcal/mol and Ki 2.93 μM, and quercetin with ΔG -7.54 kcal/mol and Ki 2.97 μM. Both compounds are anticipated to have potential as promising candidates for anxiolytic drug materials targeting the Erα.

Further visualization was performed as a graphical representation stage of the molecular docking results to analyze molecular interactions including the types of interactions that occur, binding distances, atoms in the ligand bound to the receptor, and amino acid residues involved in the interaction (Thahara et al., 2022). 2D visualization provides important insights into the interactions between ligands and amino acid residues, indicating the active site on the receptor (Thahara et al., 2022). The 2D visualization results can be seen in Figure 3 and Table 5.

Table 4. Results of molecular docking of 17 compounds from green tea against the 7UJM receptor

No	Compound Name	ΔG (kcal/mol)	Ki (μM)
(+)	Clonazepam	-7.20	5.24
1.	Methyl anthranilate	-6.05	166.34
2.	N,N-Dibutylacetamide	-4.51	493.02
3.	(+)-Catechin	-7.00	7.42
4.	(+)-Epicatechin	-7.55	2.93
5.	(-)-Catechin	-7.42	3.64
6.	(-)-Epiafzelechin	-7.18	5.50
7.	Ampelopsin	-6.83	9.93
8.	Cyanidin	-7.44	3.51
9.	Delphinidin chloride	-7.42	3.64
10.	Dihydroquercetin	-7.12	6.09
11.	Pollenitin	-6.94	8.18
12.	Quercetin	-7.54	2.97

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13.	Tricetinidin	-7.47	3.32
14.	3-O-Caffeoylquinic acid	-6.23	26.93
15.	Peonidin chloride	-7.34	4.14
16.	Malvidin	-6.73	11.65
17.	Luteoliflavan	-7.52	3.07

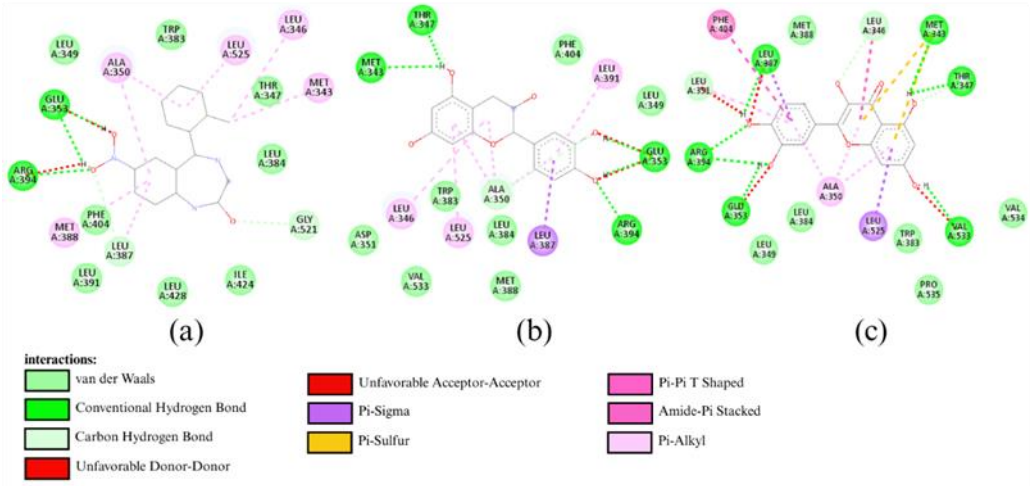

Figure 3. 2D visualization (a) clonazepam, (b) epicatechin, (c) quercetin

Table 5. Ligand interactions with 7UJM receptor amino acid residues

Compound Name	Hydrogen Bond	Hydrogen bond distance (Å)	Hydrophobic Bond
Clonazepam	**Glu353**	1.79	Leu387, Gly521, Leu346, Leu525,
	Arg394	2.20	Ala350, Met343, Met388
(+)-Epicatechin	Met343	2.83	Leu346, Ala350, Leu387, Leu391,
	Thr347	2.05	Leu525
	Glu353	1.74	
	Arg394	1.97	
Quercetin	Met343	2.75	Met343, Leu346, Ala350, Leu391,
	Thr347	2.05	Phe404, Leu525
	Glu353	1.71	
	Leu387	2.00	
	Arg394	2.45	
	Val533	2.98	

Parameters considered in the visualization stage include amino acid residue contacts, particularly the presence of hydrogen bonds that indicating interaction stability. The more hydrogen bonds present, the better and more stable the interaction between the compound ligand and amino acid residues on the receptor (Mardianingrum et al., 2022). Hydrogen bonds are considered stable and strong when they are within a distance of < 2.7 Å, because distances exceeding 2.7 Å are deemed weak and easily disrupted (Thahara et al., 2022). Therefore, based on the visualization results, quercetin is predicted to have more hydrogen bonds at qualifying distances and to exhibit compatibility with amino acid residues comparable to natural ligands and clonazepam.

In this study, the Erα is known to be a ligand-binding domain with an active site on helix 12 (Lv et al., 2022). Compounds are said to have agonistic properties if they interact through hydrogen bonding with His524 that causing helix 12 open and bind with coactivators (Mardianingrum et al., 2022). (+)-epicatechin and quercetin are predicted to be antagonistic to the Erα because they do not form hydrogen bonds with His524.

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Molecular dynamic analysis and simulation

Molecular Dynamics (MD) simulation is a method that integrates techniques from physics, mathematics, and chemistry to study protein movement processes by tracking protein conformations over time (Lv et al., 2022). The RMSD graph in Figure 4 (a) shows conformational changes of three protein complexes over 100 ns (100,000 ps) for clonazepam, (+)-epicatechin and quercetin. Based on analysis of the graph, quercetin that represented by the green trendline, appears stable with RMSD around 3-3.5 Å and low fluctuations starting at 80 ns (80,000 ps) until the end of the simulation which indicating attainment of dynamic equilibrium in the protein complex. Compared to clonazepam and (+)-epicatechin which exhibit unstable fluctuation movements, quercetin is predicted to have better interaction potential with the protein molecule and selected as the top candidate compound. Meanwhile average, minimum, and maximum RMSD values during the 100 ns (100,000 ps) MD simulation can be observed in Table 6.

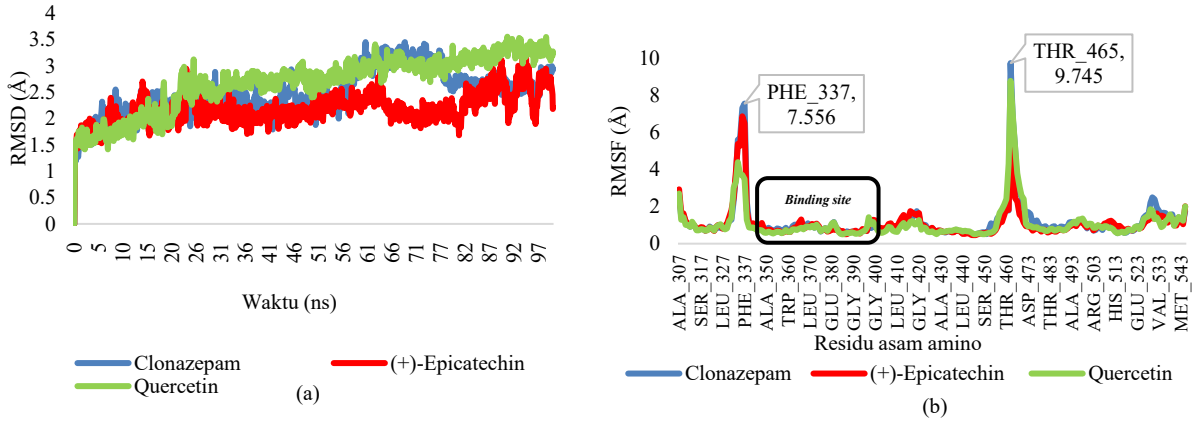

Figure 4. (a) RMSD graph, (b) RMSF graph

Table 6. Table of RMSD values during MD simulation

Complex	RMSD (Å)		
	Average	Minimum	Maximum
Clonazepam	2.48	1.13	3.45
(+)-Epicatechin	2.19	1.30	3.29
Quercetin	2.69	1.14	3.56

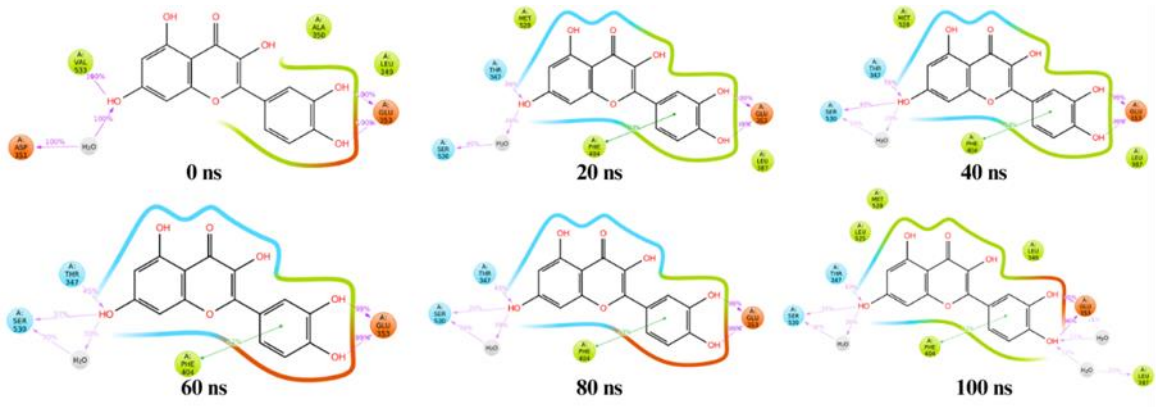

Figure 5. Conformational changes of quercetin compounds during MD simulations

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Overall, the fluctuation movements exhibited by the test compounds and reference drugs based on the RMSF graph in Figure 4 (b) appear nearly identical. Residues Thr465 and Phe337, which showed the highest fluctuations, are predicted not to be active sites of the Erα due to their significant positional changes during the MD simulation. Quercetin exhibits the lowest fluctuation at residue Glu353 with an RMSF value of 0.54 Å and at amino acid residue Arg394 with an RMSF value of 0.65 Å, which are binding sites of the 7UJM receptor. The higher stability of these two residues indicates that quercetin interacts more stably and strongly with the Erα compared to clonazepam and (+)-epicatechin.

From Figure 5, it can be observed that the conformational changes in quercetin during the MD simulation are accompanied by alterations in its contacts with amino acid residues. The conformation at 100 ns shows the best stability, as it exhibits similarity with the amino acids from the molecular docking results. The presence of more identical amino acids before and after the MD simulation indicates that the compound is stable and resistant to thermodynamic changes. The amino acid residues with the highest similarity are Glu353, Thr347, Leu387, and Phe404.

Quercetin is an abundant flavonoid in nature, found in various plants, fruits, and vegetables such as onions, cabbage, tea, apples, nuts, and berries (Hasan et al., 2022). Quercetin is also known as 3,3',4',5,7-pentahydroxyflavone, characterized chemically as an aglycone or glycoside bound to sugars like glucose, rhamnose, or rutinose (Hasan et al., 2022).

The biosynthesis of quercetin begins with the conversion of 4-coumaroyl-CoA, which is condensed by Chalcone Synthase (CHS) with three molecules of malonyl-CoA to produce naringenin (Hasan et al., 2022). The closure of the heterocyclic C ring is catalyzed by Chalcone Isomerase (CHI), resulting in naringenin. Then, with the assistance of Naringenin 3-Dioxygenase (N3DOX), naringenin produces dihydrokaempferol, which is then converted into quercetin through the action of Flavonol Synthase 1 (FLS1). Dihydrokaempferol also serves as a substrate for Flavonoid 3'-Hydroxylase (F3'H), producing dihydroquercetin, which is ultimately converted into quercetin by Flavonol Synthase 1 (FLS1) (Marín et al., 2018).

The MMGBSA calculations indicated that the Clonazepam and Quercetin complexes had nearly identical total binding free energy (ΔGTOTAL) values, at -29.2558 kcal/mol and -29.1441 kcal/mol, respectively. Despite significant differences in electrostatic energy (EEL) and solvation contributions (EGB), where Clonazepam had a positive EEL and negative EGB, while Quercetin showed the opposite, the substantial contribution from Van der Waals (VdW) interactions dominated, bringing the total stability of both complexes closer. This indicated that the VdW energy component had the most significant impact on the system (Mardianingrum et al., 2022).

Table 7. Calculation results of bond energy method of molecular mechanics-generalized born surface area (MM-GBSA)

Energy Component (kcal/mol)	Clonazepam	Quercetin
Van der Waals Interaction (VdW)	-37.7244	-36.5924
Electrostatic Energy (EEL)	106.1903	-131.2730
Electrostatic Contribution to Solvation Free Energy (E_{GB})	-93.6150	142.5944
Non-Polar Contribtio to Solvation Free Energy (E_{SURF})	-4.1067	-3.8731
ΔG_{gas} (VdW + EEL)	68.4659	-167.8654
ΔG_{solv} (E_{GB} + E_{SURF})	-97.7217	138.7213
ΔG_{TOTAL} (VdW + EEL + E_{GB} + E_{SURF})	-29.2558	-29.1441

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CONCLUSION

This study identified quercetin as a promising anxiolytic drug material through the inhibition of Erα using *in silico* studies. Screening results revealed that quercetin is non-carcinogenic, non-hepatotoxic, easily absorbed, evenly distributed, non-interfering with CYP2D6 enzyme metabolism, and potentially effective as an oral drug. Molecular docking analysis showed that quercetin has a ΔG value of -7.54 kcal/mol and a Ki of 2.97 μM, which are better than the reference drug with a ΔG value of -7.20 kcal/mol and Ki of 5.24 μM. Quercetin also demonstrated more stable interactions with the ERα binding site, indicated by Glu353 and Arg394 amino acid in RMSD and RMSF analysis during molecular dynamic simulations.

This study is limited by its *in silico* nature, which requires further validation through in vivo experiments. Future studies will focus on confirming quercetin's anxiolytic effects in animal models and exploring its formulation into a suitable delivery system. The findings of this study support the traditional use of green tea for mental health and may pave the way for its development into a standardized phytopharmaceutical.

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AUTHOR CONTRIBUTIONS

Conceptualization: H.N.A., R.M., S.; Methodology: H.N.A., R.M., S.; Software, (N/A); Validation, H.N.A., R.M., S.; Formal Analysis, H.N.A.; Investigation, H.N.A.; Resources, R.M., S.; Data Curration: H.N.A.; Writing - Original Draft, H.N.A.; Writing - Review and Editing, H.N.A., R.M., S.; Visualization, H.N.A.; Supervision, R.M., S.; Project administration, R.M., S.; Funding Acquisition, No funding was received for this research.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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